Am. J. Hum. Genet. 59:579–590, 1996

mtDNA Polymorphism in East Asian Populations, with Special Reference to the Peopling of Japan

Satoshi Horai,[1] Kumiko Murayama,[1] Kenji Hayasaka,[1] Satoe Matsubayashi,[1] Yuko Hattori,[1] Goonnapa Fucharoen,[2] Shinji Harihara,[3] Kyung Sook Park,[4] Keiichi Omoto,[5] and I-Hung Pan[6]

[1]Department of Human Genetics, National Institute of Genetics, Mishima, Shizuoka, Japan; [2]Khon Kaen University, Khon Kaen, Thailand; [3]Department of Anthropology, Graduate School of Science, University of Tokyo, Tokyo; [4]Department of Biology, Sung-Shin Women's University, Seoul; [5]International Center for Japanese Studies, Kyoto; and [6]National Taiwan University, Taipei

Summary

Nucleotide sequences of the major noncoding (D-loop) region of human mtDNA from five East Asian populations including mainland Japanese, Ainu, Ryukyuans, Koreans, and Chinese were analyzed. On the basis of a comparison of 482-bp sequences in 293 East Asians, 207 different sequence types were observed. Of these, 189 were unique to their respective populations, whereas 18 were shared between two or three populations. Among the shared types, eight were found in common between the mainland Japanese and Koreans, which is the largest number in the comparison. The intergenic COII/tRNA^{Lys} 9-bp deletion was observed in every East Asian population with varying frequencies. The D-loop sequence variation suggests that the deletion event occurred only once in the ancestry of East Asians. Phylogenetic analysis revealed that East Asian lineages were classified into at least 18 monophyletic clusters, though lineages from the five populations were completely intermingled in the phylogenetic tree. However, we assigned 14 of the 18 clusters for their specificity on the basis of the population from which the maximum number of individuals in each cluster was derived. Of note is the finding that 50% of the mainland Japanese had continental specificity in which Chinese or Koreans were dominant, while <20% of either Ryukyuans or Ainu possessed continental specificity. Phylogenetic analysis of the entire human population revealed the closest genetic affinity between the mainland Japanese and Koreans. Thus, the results of this study are compatible with the hybridization model on the origin of modern Japanese. It is suggested that ~65% of the gene pool in mainland Japanese was derived from the continental gene flow after the Yayoi Age.

Received January 3, 1996; accepted for publication June 25, 1996.

Address for correspondence and reprints: Dr. Satoshi Horai, Department of Human Genetics, National Institute of Genetics, Yata 1,111, Mishima, Shizuoka, 411 Japan. E-mail: shorai@lab.nig.ac.jp

0002-9297/96/5903-0013$02.00

Introduction

There have been at least two major migration events that brought immigrants from the Asian continent to the Japanese archipelago. The Jomon people arrived in Japan >12,000 years ago, although the exact timing and geographic origin of this migration event are still puzzling (Turner 1990; Nei 1995). The Yayoi people, originally from northeast Asia, started migrating to Japan from the Korean peninsula ~2,300 years ago. One of major issues in the Japanese prehistory is concerned with the extent to which the aboriginal Jomon and the more recently migrating Yayoi contributed genetically to modern Japanese populations (Chard 1974, p. 114). There are two contemporary ethnic groups that appear to be distinct from the mainland Japanese populations living on the centrally located islands of Honshu, Shikoku, and Kyushu. They are the Ainu, inhabiting the northern island of Hokkaido, and the Ryukyuans, living in the southernmost island of Okinawa. There is some agreement that these two groups represent modern descendants of the aboriginal people of the Neolithic Jomon Age (Hanihara 1991; Omoto 1992; Kozintsev 1993).

There are several theories on the origins of modern Japanese populations, some of which were proposed >100 years ago (reviewed by Mizoguchi 1986). These theories attempt to explain a fairly large range of morphological, cultural, and genetic variation represented in modern Japanese populations and recognized in archaeological and fossil evidence. Currently, hypotheses can be classified into two major groups: hybridization and transformation theories. Hybridization theories claim that modern Japanese are the result of an admixture between different immigrant populations and predict that modern Japanese have genes deriving from both the Jomon and Yayoi people. One of the hybridization theories is the so-called dual structure model for the population history of Japanese (Hanihara 1987, 1991, 1992). In this hypothesis, the elemental population involved in the formation of the Japanese population may have been the Jomon people, who are supposed to have originated in Southeast Asia. Migrants after the Yayoi Age, whose homeland was probably in northeast Asia, gradually mixed with the preexistent Jomon people in Japan. On the other hand, most transformation theories posit that the people of Japan gradually evolved from a single ancient population that migrated from southern China at the end of Pleistocene (Hasebe 1940; Suzuki 1969). They formed the Jomon people, who occupied the entire archipelago of Japan during the Jomon Age (12,000–2,300 years ago). The Jomon people then gradually transformed their morphological characteristics to form the modern Japanese, who are now living on the main islands of Japan (Suzuki 1981; Mizoguchi 1986). In this theory, migrants after the Yayoi Age might have contributed culturally but not genetically to the formation of modern Japanese. However, these theories on the origins of the modern Japanese were primarily based on morphological evidence. Therefore, it is necessary to study the origin and formation of modern Japanese on the basis of genetic evidence (Hammer and Horai 1995; Nei 1995; Omoto 1995).

By virtue of its rapid rate of evolution and maternal mode of inheritance (Hutchison et al. 1974; Brown et al. 1979; Giles et al. 1980), mtDNA permits discrimination and quantification of genetic relationships among closely related individuals. Restriction enzyme analysis of mtDNA has been a powerful tool in attempts to quantify evolutionary relationships among human ethnic groups (Horai et al. 1986; Cann et al. 1987; Wallace 1995 and references therein). Although restriction enzyme analyses represent polymorphisms over the whole mitochondrial genome, some ambiguities remain with respect to the actual number of nucleotide differences and the estimation of genetic distances (Kocher et al. 1989). In recent mtDNA analyses, it has become popular to employ direct sequencing by means of a PCR. The target is a major noncoding (D-loop) region of mtDNA that has apparently evolved several times faster than other parts (Vigilant et al. 1989, 1991; Horai and Hayasaka 1990; Horai et al. 1991, 1993; Di Rienzo and Wilson 1991). Sequence analysis of this region therefore offers high resolution that discriminates among even very closely related individuals.

To investigate the peopling of Japan and to create the most appropriate model for the origins of modern Japanese, we examined the mtDNA sequence variation in the D-loop region from five East Asian populations: the mainland Japanese, Ryukyuans, Ainu, Koreans, and Chinese. These results are discussed in light of sequence evolution and shared identical sequences among populations in East Asia. We also examined in detail the evolution of mtDNA sequences at the worldwide population level.

Material and Methods

Samples of East Asians

Blood samples were obtained from 66 Chinese in Taiwan, 64 Koreans in South Korea, 50 Ryukyuans in Okinawa, and 51 Ainu in Hokkaido. Many of the Chinese who inhabit Taiwan are known to be direct descendants of migrants from mainland China after the seventeenth century. Moreover, the present Chinese samples do not include those from Taiwanese aboriginal populations who show linguistic and physical affinity to the Austronesian-speaking populations of Southeast Asia (Bellwood et al. 1995). For the Ainu subjects, restriction enzyme analysis of the same samples was reported by Harihara et al. (1988). Genomic DNA was prepared from the buffy coat in blood through treatment with SDS and proteinase K and subsequent phenol/chloroform extraction.

Direct Sequencing of PCR Products

A fragment of mtDNA was amplified by means of the PCR method described by Saiki et al. (1988). A set of nonbiotinylated and 5′-bitionylated primers (A and E primers; see below) were designed to amplify a DNA fragment from the D-loop region of mtDNA. PCR was carried out under the following thermal cycle conditions: incubation at 94°C for 15 s, 45°C for 15 s, 72°C for 30 s, for a total of 30 cycles. To generate single-stranded template DNAs from PCR products, the PCR products were mixed with magnetic beads (Dynabeads M-280 Streptavidin; Dynal). Both strands were then prepared as single-stranded DNAs by using alkali denaturation and magnetic separation as described in the manufacturer's protocol and employed as templates for sequencing reactions. The following oligonucleotide primers were designed for amplification and sequencing: A: 16011-5′-AAACTATTCTCTGTTCTTTC-3′-16030; B: 16204-5′-AGCAAGTACAGCAATCAACC-3′-16223; C: 16403-5′-CCATCCTCCGTGAAATCAAT-3′-16423; D: 60-5′-AAAATACCAAATGCATGGAG-3′-41; E: 181-5′-TAATATTGAACGTAGGTGCG-3′-162. (The notation of Anderson et al. (1981) is used for numbering of bases.)

Detection of the COII/tRNA^{Lys} Intergenic Deletion

A set of oligonucleotide primers was designed as follows, to amplify a COII/tRNA^{Lys} intergenic region of mtDNA: 8211-5′-TCGTCCTAGAATTAATTCCC-3′-8230 and 8310-5′-AGTTAGCTTTACAGTGGGCT-3′-8291. The amplified fragments were separated byelectrophoresis on 4% agarose gels and detected fluorographically after staining with ethidium bromide.

Data Analyses

The number of nucleotide substitutions per site between individual sequences was estimated using the two-parameter model of nucleotide substitutions (Kimura 1980). On the basis of the estimated number of nucleotide substitutions, phylogenetic trees were constructed using the neighbor-joining (NJ) method (Saitou and Nei 1987) and the unweighted pair-group method with arithmetic mean (UPGMA) (Nei 1987).

Table 1

Unique and Common mtDNA Sequence Types Observed in Five East Asian Populations

				NO. OF COMMON TYPES SHARED BETWEEN	
POPULATION	NO. OF INDIVIDUALS	NO. OF SEQUENCE TYPES	NO. OF UNIQUE TYPES (% individuals)[a]	Two Populations (counterpart)[b]	Three Populations (companion)[c]
Chinese	66	58	54 (94)	3 (K2, A1)	1 (c)
Korean	64	49	36 (64)	9 (J5, C2, R1, A1)	4 (a, b, c, d)
Mainland Japanese	62	53	41 (71)	9 (K5, A3, R1)	3 (a, b, d)
Ryukyuan	50	44	40 (86)	2 (J1, K1)	2 (a, b)
Ainu	51	25	18 (67)	5 (J3, K1, C1)	2 (c, d)

[a] Percent number of individuals who possess unique types in each population.

[b] Single capital letters refer to abbreviations of population as follows: C = Chinese; K = Korean; J = mainland Japanese; R = Ryukyuan; and A = Ainu. Numbers of common types are indicated by suffixes to abbreviations of population.

[c] Four common types are shared by three populations and designated a through d. Populations appeared in each type are also given by single capital letter abbreviations as follows: a and b: KRJ; c: KAC; d: JAK.

Results and Discussion

Sequence Differences and Identities in East Asians

We determined the nucleotide sequence of a 482-bp fragment of the D-loop region (positions 16129–16569 followed by positions 1–41 in the reference sequence of Anderson et al. 1981) for 231 East Asians from four local populations (Koreans, Chinese, Ainu, and Ryukyuans). Together with the published sequence data for 62 mainland Japanese from Shizuoka Prefecture in central Japan (Horai and Hayasaka 1990; Horai et al. 1991), a total of 293 sequences from East Asians were analyzed. There were 207 distinct types of sequences in total. Of these, 189 were unique to their respective populations, whereas 14 were shared between two populations and 4 were found in common among three populations. The number of these sequence types and of unique types observed in the five Asian populations are shown in table 1. The smaller number of sequence types observed in the Ainu was a result of the many identical sequences in that population. In fact, the most frequent sequence type was shared by eight Ainu individuals, and the second most frequent type was observed in seven individuals of the Ainu. Among 14 sequence types shared between two populations, 5 were found in common between the Koreans and mainland Japanese, 3 between the mainland Japanese and Ainu, 2 between the Chinese and Koreans, and 1 each in four population pairs: the Chinese and Ainu, the Koreans and Ryukyuans, the Korean and Ainu, and the mainland Japanese and Ryukyuans. We identified four types shared among three populations. There were two different types shared among the Koreans, mainland Japanese, and Ryukyuans. Two others were found in common among the Chinese, Koreans, and Ainu and among the Koreans, mainland Japanese and Ainu, respectively.

The number of shared types observed between each pair of two populations is summarized and shown in figure 1. It should be noted that the mainland Japanese and Koreans have eight different sequence types in common, and these types were shared between 14 mainland Japanese (23% of the population) and 17 Koreans (27% of the population). The mainland Japanese also share four sequence types with the Ainu, while the number of shared types is less than three in the rest of the comparisons between two populations. As shown in figure 1, 10 Ainu shared three common types with four Koreans.

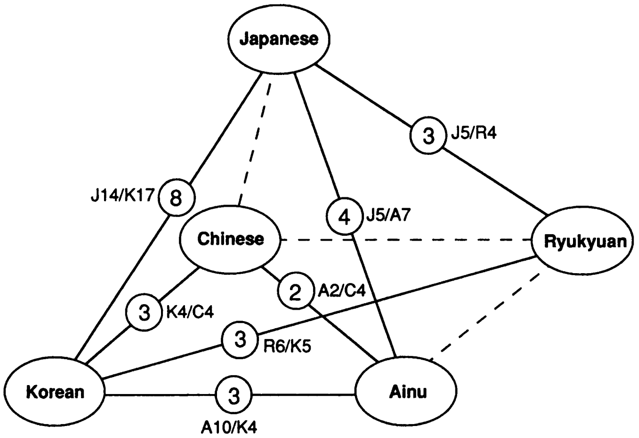

Figure 1 Number of shared types observed between each pair of two populations. The figure in a circle on the solid line connecting two populations is the number of shared types. Pairs of populations that have no shared types are shown by dashed lines. Single capital letters refer to the abbreviations of population as follows: C = Chinese; K = Korean; J = mainland Japanese; R = Ryukyuan; and A = Ainu. The figures following the population abbreviation shown beside the circles are total number of individuals sharing types from the respective two populations.

However, this does not necessarily indicate a close relationship between the Ainu and Koreans, since one of the three common types happened to be shared by eight Ainu and only one Korean. It is worth noting that the Ainu and Ryukyuans did not possess any sequence types in common, though these two populations are considered to be direct descendants of the Jomon people (discussed in Relationships between the Ainu and Ryukyuans).

In addition to the above East Asian data, we analyzed identical sequence pairs together with sequence data for 23 Africans (Horai and Hayasaka 1990; Vigilant 1990), 20 Europeans (Horai and Hayasaka 1990), and 72 Amerinds (Horai et al. 1993). Each of three human populations exhibited unique sequences and did not show any shared sequence types with other populations (data not shown). The presence of shared sequence types between two populations should reflect either a closer genetic relationship or recent migrations between the two populations. Since, in terms of shared types, the Ainu and Ryukyuans (descendants of the aboriginal Jomon people) showed a lesser degree of genetic affinity to the Koreans than did the mainland Japanese, one explanation may be that a relatively large number of common types observed between mainland Japanese and Koreans may have resulted from recent migrations via the Korean peninsula.

Phylogeny of mtDNA Sequences

The 482-bp sequences from a total of 293 individuals from East Asia were aligned and compared, and the number of nucleotide substitutions between each pair of sequences was estimated. Nucleotide diversity among the 293 East Asian was estimated to be 1.34%, which is three to four times higher than the corresponding value estimated from restriction enzyme analysis of the entire mtDNA genome (Brown 1980; Horai et al. 1986; Cann et al. 1987). By using the estimated number of nucleotide substitutions between individual sequences, a phylogenetic tree was constructed using the NJ method (Saitou and Nei 1987), as shown in figure 2. On the basis of branching patterns in the tree, sequences were classified into 18 monophyletic clusters, which are shown by brackets with clustering numbers (C1–C18). To ensure robustness of these clusters, we examined shared polymorphic sites within each cluster, as shown in table 2. The majority of members in each cluster shared a specific combination of two to five polymorphic sites, with exceptions of members in C3 and C13, who shared only one polymorphic site. A C-to-T transition at 16223 (nucleotide position in the reference sequence of Anderson et al. 1981) was shared by most members (212 of the 217 individuals) in clusters C5 through C18, while this polymorphism was virtually absent in clusters C1 through C4. Furthermore, in some clusters, all members exhibited a specific polymorphism that was virtually absent in other clusters. For example, a T-to-C transition at 16304 was observed in all members of C4, which was unique to this cluster and was completely absent in other clusters. Unique polymorphisms were also seen in ≥10 other clusters. Therefore, it is likely that most of the clusters could reflect their ancestral states of lineage divergence. However, it is apparent that lineages from the five Asian populations were completely intermingled in the phylogenetic tree, though individuals from single populations dominated in some of the clusters.

To evaluate the features of clustering patterns further, the composition of the 18 clusters and geographic distribution of the 293 East Asian lineages were summarized, as shown in table 3. Although the numbers of individuals sampled from the five populations were not the same, we assigned "specificity" for each cluster on the basis of the population from which the maximum number of individuals was derived. In this way, we were able to assign specificity for 14 of the 18 clusters. Although this assignment of specificity seems to be somewhat arbitrary, the rule for assignment is a simple "majority-rules" voting procedure. Furthermore, it may be useful for understanding the relationships of mtDNA sequences among very closely related human populations such as East Asians because ancient migrations between adjacent geographic areas are anticipated. For example, in C1, 10 Ainu individuals appeared together with only 3 members each from the mainland Japanese, Koreans, and Chinese. We therefore named this cluster Ainu-1 (table 3). We could not assign specificity for four clusters, since there were the same number of individuals from more than two populations: they were C6, C7, C11, and C12. It is interesting to note that three individuals from mainland Japan were dominant only in a small cluster (C8), and most of the other Japanese were included under other specificity. However, Chinese exhibited four dominant clusters (C2, C4, C9, and C18) and Koreans did three (C5, C10, and C14). On the other hand, the Ainu exhibited two dominant clusters (C1 and C16), and the Ryukyuans showed four dominant clusters (C3, C13, C15, and C17). Table 3 also shows the assigned specificity for the sum of individuals from each of five localities in East Asia. It is surprising that 25.8% of mainland Japanese showed Chinese specificity and 24.2% of them exhibited Korean specificity. Thus, 50% of mainland Japanese had continental specificity (both the Chinese and Koreans). By contrast, 9.8% of the Ainu showed both Chinese and Korean specificity. Ryukyuans showed almost the same breakdown in specificity (10% Chinese and 8% Korean). It is relevant to note that one third (17 individuals) of the Ainu exhibited the two dominant clusters (C1 and C16), and 50% of the Ryukyuans showed Ryukyuan specificity, indicating unique phylogenetic affiliation for the two ethnic groups of Japan.

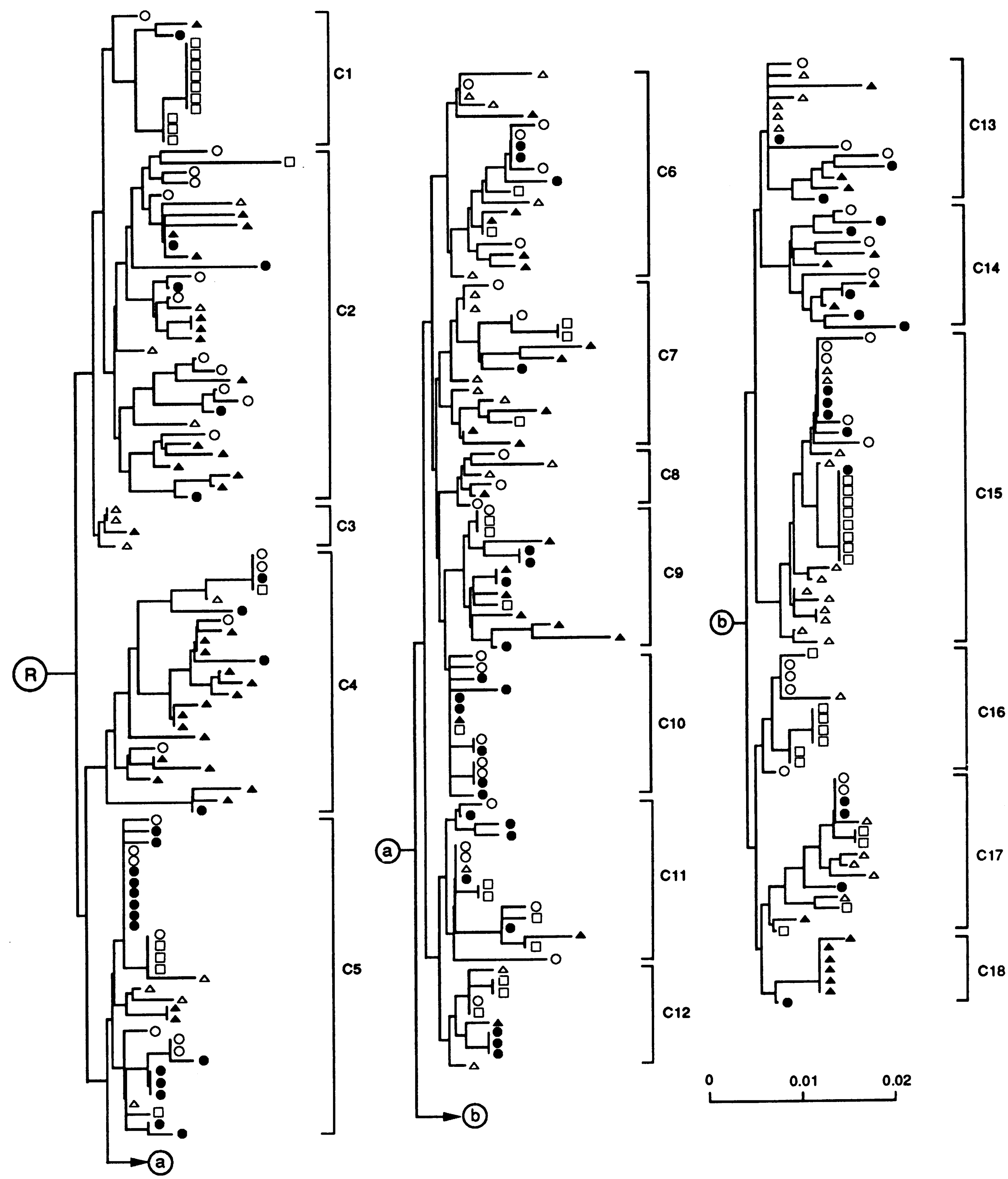

Figure 2 Phylogenetic tree of 293 mtDNA lineages from five populations of East Asians. On the basis of clustering patterns in the tree, all lineages were classified into 18 monophyletic clusters, which are shown by brackets with cluster numbers C1–C18. The symbols at the tip of each branch indicate local populations: mainland Japanese (○), Ryukyuan (△), Ainu (□), Korean (●), and Chinese (▲). Nucleotide substitutions per site are indicated on the scale below the tree.

COII/tRNA^{Lys} Intergenic 9-bp Deletion

The presence of a 9-bp deletion in the COII/tRNA^{Lys} intergenic region of mtDNA is one of the characteristics not only of Asians (Horai and Matsunaga 1986; Horai et al. 1987; Stoneking and Wilson 1989; Horai 1991*a*, 1991*b*; Ballinger et al. 1992; Harihara et al. 1992; Passarino et al. 1993) but also of populations of Asian origin, including Polynesians (Hertzberg et al. 1989; Hagelberg and Clegg 1993; Lum et al. 1994; Redd et al. 1995) and Native Americans (Schurr et al. 1990; Ward

Table 2

Shared Polymorphic Sites in the 18 Clusters

Cluster	No. of Lineages	Shared Polymorphic Sites within a Cluster[a]				
C1	13	16189:T→C(11)	16231:T→C(13)	16266:C→T(12)	16292:C→T(7)	16519:T→C(12)
C2	34	9-bp deletion(34)	16189:T→C(34)	16217:T→C(19)	16519:T→C(33)	...
C3	4	16519:T→C(4)	...	...	...	...
C4	25	16129:G→A(16)	16162:A→G(8)	16172:T→C(13)	16304:T→C(25)	16519:T→C(17)
C5	31	16223:C→T(31)	16362:T→C(18)	16519:T→C(30)	...	...
C6	20	16223:C→T(19)	16290:C→T(14)	16319:G→A(20)	...	...
C7	16	16223:C→T(16)	16278:C→T(10)	16362:T→C(14)	16227:A→G(6)	16234:C→T(7)
C8	6	16223:C→T(6)	16390:G→A(6)	16362:T→C(4)	16519:T→C(4)	...
C9	14	16223:C→T(14)	16311:T→C(14)	16362:T→C(14)	...	...
C10	14	16223:C→T(14)	16362:T→C(14)	...	...	...
C11	16	16189:T→C(14)	16223:C→T(14)	16362:T→C(16)	...	...
C12	10	16189:T→C(10)	16223:C→T(10)	16519:T→C(9)	...	...
C13	14	16223:C→T(14)	...	...	...	...
C14	12	16223:C→T(12)	16298:T→C(12)	16519:T→C(6)	...	...
C15	30	16209:T→C(21)	16223:C→T(28)	16324:T→C(29)	16291:C→T(10)	...
C16	12	16129:G→A(12)	16223:C→T(12)	16519:T→C(11)	...	...
C17	15	16129:G→A(14)	16189:T→C(12)	16223:C→T(15)	16297:T→C(10)	16298:T→C(11)
C18	7	16129:G→A(7)	16223:C→T(7)	16192:C→T(7)	16297:T→C(6)	...

[a] Each polymorphic site corresponds to the following site and replacement in the reference sequence reported by Anderson et al. (1981). Number in parenthesis represents number of lineages that exhibited the polymorphism.

et al. 1991, 1993; Shields et al. 1992, 1993; Torroni et al. 1992; Horai et al. 1993). However, some studies have suggested multiple origins for the deletion in Asia (Schurr et al. 1990; Ballinger et al. 1992; Torroni et al. 1994; Redd et al. 1995). We screened the present 293 East Asians from five localities for the 9-bp deletion. The frequency of the 9-bp deletion was 20% for Chinese, 18% for mainland Japanese, 8% for Koreans, 8% for Ryukyuans, and 2% for Ainu. A total of 34 East Asians exhibited the 9-bp deletion, and all of them were included in the C2 cluster in the phylogenetic tree based on D-loop sequences (fig. 2). This indicates that the deletion event occurred once in the ancestry of East Asian lineages. Furthermore, since there were at least five subclusters in C2 and the nucleotide diversity within C2 was the highest (table 3), the deletion event appears to have occurred a relatively long time ago. Recently, Horai et al. (1995) analyzed complete mtDNA sequences from three humans (African, European, and Japanese) and four species of hominoids in order to infer modern human origins. On the basis of these data for humans, we estimated the substitution rate for the 482-bp segment as 8.6×10^{-8}/site/year. The average divergence for the coalescence of the cluster containing the 9-bp deletion was 1.285% (table 3). Assuming the average rate of the D-loop sequence substitution of 8.6×10^{-8}/site/year yields a mean estimate of 75,000 years for the coalescence of the 9-bp deletion cluster.

mtDNA Polymorphism and the Peopling of Japan

As mentioned in the Introduction, there are two major hypotheses on the origins of mainland Japanese. Patterns of mtDNA sequence variation can be used to test these hypotheses from the perspective of maternal lineages. On the basis of the analysis of identical sequence pairs shared between populations, it was revealed that the mainland Japanese had sequence types in common more frequently with Koreans than with the Ainu and Ryukyuans (table 1 and fig. 1). Furthermore, on the basis of clustering analysis in the phylogenetic tree, it can be inferred that the continental specificity in mainland Japanese (50%) were much more abundant than for either the Ainu or Ryukyuans (average value 19%). These findings indicate that a great quantity of maternal lineages was introduced into Japan by immigrants from the Korean peninsula after the Yayoi Age. These results lend support to the hybridization theory on the origin of mainland Japanese. The transformation theory contends that genetic variation in mainland Japanese derives solely from their Jomon ancestors and does not reflect a Yayoi admixture (Suzuki 1981; Mizoguchi 1986). Since the mtDNA sequence polymorphism found indicates that the mainland Japanese have been considerably influenced by a continental gene flow, the transformation theory obviously is incompatible with the present results. Recently, Nei (1995) used evidence from a survey of 18 protein polymorphic loci in mainland Japanese, Ainu, Ryukyuans, and their surrounding populations to question the validity of the hybridization theory of Japanese origins. A phylogenetic tree indicated that the Ainu and Ryukyuans are closest to each other and are also closely allied with the mainland Japanese and Koreans. However, the three Japanese populations were

Table 3

Composition of the 18 Clusters, and Geographic Distribution of 293 East Asian Lineages

		NO. OF LINEAGES FROM					
CLUSTER	SPECIFICITY	Mainland Japan	Okinawa	Hokkaido Ainu	Korea	Taiwan Chinese	NUCLEOTIDE DIVERSITY (%)
C1	Ainu-1	1	0	10	1	1	.357
C2	Chinese-1	11	4	1	5	13	1.285
C3	Ryukyuan-1	0	3	0	0	1	.207
C4	Chinese-2	4	1	1	4	15	1.275
C5	Korean-1	7	4	4	14	2	.514
C6	Unassigned-1	5	5	2	3	5	.716
C7	Unassigned-2	2	5	3	1	5	1.204
C8	Japanese-1	3	2	0	0	1	.542
C9	Chinese-3	1	0	3	4	6	.743
C10	Korean-2	5	0	1	7	1	.309
C11	Unassigned-3	5	1	4	5	1	.703
C12	Unassigned-4	1	2	3	3	1	.407
C13	Ryukyuan-2	3	5	0	3	3	.739
C14	Korean-3	3	0	0	5	4	1.009
C15	Ryukyuan-3	5	12	8	5	0	.403
C16	Ainu-2	4	1	7	0	0	.352
C17	Ryukyuan-4	2	5	4	3	1	.618
C18	Chinese-4	0	0	0	1	6	.178
	Total	62	50	51	64	66	1.336
	Sum of specificity:						
	Chinese	16 (25.8)	5 (10.0)	5 (9.8)	14 (21.9)	40 (60.6)	
	Korean	15 (24.2)	4 (8.0)	5 (9.8)	26 (40.6)	7 (10.6)	
	Japanese	3 (4.8)	2 (4.0)	0 (0)	0 (0)	1 (1.5)	
	Ainu	5 (8.1)	1 (2.0)	17 (33.3)	1 (1.6)	1 (1.5)	
	Ryukyuan	10 (16.1)	25 (50.0)	12 (23.5)	11 (17.4)	5 (7.6)	
	Unassigned	13 (21.0)	13 (26.0)	12 (23.5)	12 (18.8)	12 (18.2)	

NOTE.—Numbers in parentheses are percentages.

quite distinct from contemporary Southeast Asians in the phylogenetic tree. This does not support a hypothesis based on morphological characteristics, which states the ancestors of the Ainu and Ryukyuans (Jomon people) are the direct descendants of Paleolithic Southeast Asians (Turner 1976, 1990; Hanihara 1991). Nei (1995) favors a modification of the transformation theory (called the "out-of-northeast-Asia hypothesis") that posits: (1) modern human first entered into Japan ~30,000 years ago from northeast Asia; (2) an occasional gene flow from northeast Asia continued until 12,000 years ago, when the Japanese archipelago disconnected from the Asian continent; and (3) the Yayoi migration, while making a large cultural contribution, had little influence on the gene pool of modern Japanese.

The mtDNA polymorphisms discussed in this paper provide a maternally based test of one aspect (3) of the out-of-northeast-Asia hypothesis. It is possible to estimate the proportion of mtDNAs derived from the Yayoi migration if it is assumed that present-day frequencies of continental specificity in Ainu/Okinawa and Korea/China are similar to frequencies in the Jomon and Yayoi populations, respectively (Reed 1969). Assuming that population mixture is the only process affecting the system, if q_{AO} is the frequency of continental specificity in Jomon ancestor of modern Japanese (19%; see table 3), q_{KC} is the frequency of continental specificity in the Yayoi ancestors (67%), and q_M is the frequency of continental specificity in mainland Japanese (50%), then M is the present proportion of mtDNAs derived from the Yayoi:

$$M = \frac{q_M - q_{AO}}{q_{KC} - q_{AO}} .$$

Substitution of the frequencies of continental specificity into this equation yields the following results: the proportion of mtDNAs derived from the Yayoi is 65%, and the proportion derived from the Jomon is 35%. Therefore, the mtDNA data support the hybridization hypothesis that migrations during the Yayoi Age (2,300—1,700 years ago) and the subsequent Kofun period (1,700—1,400 years ago) made a significant maternal contribution to the gene pool of modern Japanese (Yamaguchi 1982; Hammer and Horai 1995).

Relationships between the Ainu and Ryukyuans

The present mtDNA data lend support to the hybridization theory. As mentioned above, after the Yayoi Age a great quantity of migrations to Japan occurred from the Asian continent. It has been thought that people migrated first into the western part (Kyushu island) of Japan and gradually moved into the Honshu island and mixed with the preexistent Jomon people (Hanihara 1991). However, there is an idea that the migrants pushed the Jomon people into the northern and southern parts of Japan (Nei 1995). This idea assumes that the present Ainu living in Hokkaido are descendants of the Jomon people who were forced to move northward, while the Ryukyuans living in Okinawa are Jomon descendants who moved south. If this is the case, the present Ainu and Ryukyuans began to diverge from a single Jomon population just after the Yayoi migration. As shown in table 1, the Ainu and Ryukyuans did not possess any sequence types in common, though a total of 18 sequence types were shared among other populations of East Asia. As mentioned above, we estimated the substitution rate for the 482-bp segment as 8.6×10^{-8}/site/year. Applying this rate, we estimated a minimum coalescence time (T in years) when given two sequences that differ by 1 nt in the 482-bp region in the following:

$$T = 1/(482 \times 2 \times 8.6 \times 10^{-8}) = 12{,}064 \text{ years} .$$

Since all pairwise comparisons of mtDNA sequences between the Ainu and Ryukyuans showed that minimum nucleotide differences were 1, the present Ainu and Ryukyuan populations should have been separated from each other for as long as 12,000 years, if the above coalescence time is valid. The results strongly suggest that ancestral populations for the present Ainu and Ryukyuans must have existed as different populations when the Yayoi migration took place ~2,300 years ago. Analysis of shared sequence types showed that the Ryukyuans shared one type each with the mainland Japanese and Koreans, and two types each with both the mainland Japanese and Koreans (table 1). In contrast, the Ainu shared three types with the mainland Japanese and one type each with Koreans and Chinese. Furthermore, the common types shared among three populations observed in the Ainu and Ryukyuans are different. These results imply that, in the Yayoi Age, some ancestral Ryukyuans might have stayed in the western part of Japan, whereas the ancestral Ainu population already was inhabiting Hokkaido, even if some of the Ainu and Ryukyuans descended from a common ancestral Jomon stock. Differences between the Ainu and Ryukyuan are also conspicuous from the phylogenetic analysis. In the clustering analysis (table 3), the Ainu exhibited two dominant clusters (C1 and C16), of which 17 (33%) of the 51 Ainu subjects were included in the two clusters. In C1, however, no Ryukyuan appeared, while there were three lineages each from mainland Japanese, Chinese, and Korean. In C16, only one Ryukyuan appeared together with seven Ainu and four mainland Japanese. These results suggest that the Ryukyuans with Ainu specificity are quite rare. On the other hand, four Ryukyuan dominant clusters were assigned: they were C3, C13, C15, and C17. In both C3 and C13, a total of eight Ryukyuans appeared, together with three mainland Japanese, three Koreans, and four Chinese but no Ainu. However, in both C15 and C17, a total of 17 Ryukyuans (34% of the population) existed together with 12 Ainu (24% of the population), 7 mainland Japanese, 3 Koreans, and 1 Chinese. This indicates a closer genetic relationship between the Ryukyuans and Ainu who were included in groups having Ryukyuan specificity. These results lend some degree of support to the hypothesis that the Ainu and Ryukyuans exhibited closer genetic affinity as the direct descendants of the Jomon people. However, since the divergence between the Ainu and Ryukyuan populations is assumed to have taken place as long as 12,000 years ago and that only part of the Ainu and Ryukyuans share a common ancestry, it is unlikely that the ancestral populations of both the Ainu and Ryukyuans existed as a single panmictic Jomon population when the Yayoi migrations began to take place.

Nucleotide Diversity and Phylogeny in the Entire Human Population

To infer the phylogenetic positions of the five East Asian populations in the entire human population, we analyzed mtDNA sequence data for the five Asian populations together with the published data for Africans (Horai and Hayasaka 1990; Vigilant 1991), Europeans (Horai and Hayasaka 1990), and Amerinds (Horai et al. 1993). We estimated intrapopulational (d_X or d_Y), interpopulational (d_{XY}), and net (d_A) nucleotide diversity among the eight human populations (table 4). In the East Asian populations, values of nucleotide diversity within population (d_X) were 1.65% for Chinese, 1.22% for Korean, 1.32% for Japanese, 1.01% for Ryukyuan, and 1.26% for Ainu. Among all East Asians, nucleotide diversity was estimated at 1.34%, which is slightly larger than that in Amerinds (1.29%). The nucleotide diversity was the highest within Africans (2.08%) and lowest within Europeans (0.95%). Furthermore, nucleotide diversity within African was larger than that of interpopulational diversity, with the exception observed between African and Chinese. Thus, the earlier finding that, compared with Europeans and Asians, Africans have highly diversified mtDNAs, which were deduced from restriction-enzyme analysis (Horai et al. 1986; Cann et al. 1987) and extensive D-loop sequence analysis for Africans reported by Vigilant et al. (1991), was confirmed by quantitative analysis of the nucleotide sequences

Table 4

Estimates of Interpopulational (d_{xy}), Intrapopulational (d_x or d_y), and Net (d_A) Nucleotide Diversities among Eight Human Populations

Population	African ($N = 23$)	European ($N = 20$)	Chinese ($N = 66$)	Korean ($N = 64$)	Japanese ($N = 62$)	Ryukyuan ($N = 50$)	Ainu ($N = 51$)	Amerind ($N = 72$)
African	**2.081**	1.829	2.100	1.883	1.917	1.802	1.901	2.054
European	.312	**.953**	1.417	1.259	1.294	1.154	1.289	1.437
Chinese	.234	.115	**1.652**	1.472	1.509	1.393	1.520	1.646
Korean	.234	.174	.037	**1.218**	1.269	1.129	1.282	1.375
Mainland Japanese	.216	.157	.022	.000	**1.321**	1.184	1.322	1.415
Ryukyuan	.258	.174	.063	.017	.019	**1.008**	1.181	1.297
Ainu	.231	.183	.064	.044	.032	.048	**1.259**	1.445
Amerind	.367	.314	.173	.119	.108	.146	.169	**1.293**

NOTE.—All values are multiplied by 100. The figures on the diagonal refer to d_x (or d_y), and those above the diagonal d_{xy}. The figures below the diagonal represent the values of $d_A = d_{xy} - (d_x + d_y)/2$.

from the entire human population. These results lend support for a hypothesis of African origin for modern humans (Horai et al. 1995).

The d_A genetic distance (Nei and Miller 1990) matrix for the eight populations compared is also given in table 4. The larger distances were found between Africans and the other populations, and the largest value was observed between Africans and Amerinds. By contrast, the five East Asian populations showed very small distances between them, even reaching zero for the distance between Koreans and Japanese, indicating again a close genetic relationship between the two populations. This suggests that a hybridization must have occurred between the two populations. On the basis of d_A distances, phylogenetic dendrograms (figs. 3 and 4) were constructed by the NJ method (Saitou and Nei 1987), as well as by the UPGMA method (Nei 1987). In the unrooted network (fig. 3), the five East Asian populations appeared in very close proximity by leaving Africans, Europeans, and Amerinds on the outskirt of the network. This is also conspicuous in the UPGMA tree (fig. 4), in which Africans first diverged from the rest of the populations. Then, the Europeans branched off, and subsequently the Amerinds diverged. Finally, the five East Asian populations branched off and formed a monophyletic cluster (bootstrap probability 74%). In the East Asian cluster, although the initial offshoot remains unclear because of the low bootstrap probability (36%), the Ainu first diverged, followed by the subsequent divergences of Chinese, Ryukyuans (72%), and Korean-Japanese clade (88%). Major features in the phylogenetic analyses shown in figure 3 and figure 4 generally agree with the phylogenetic relationships of human populations obtained by the protein polymorphism (Nei and Livshits 1989; Nei and Roychourdhury 1993) as well as those revealed by the microsatellite DNA polymorphism (Bowcock et al. 1994).

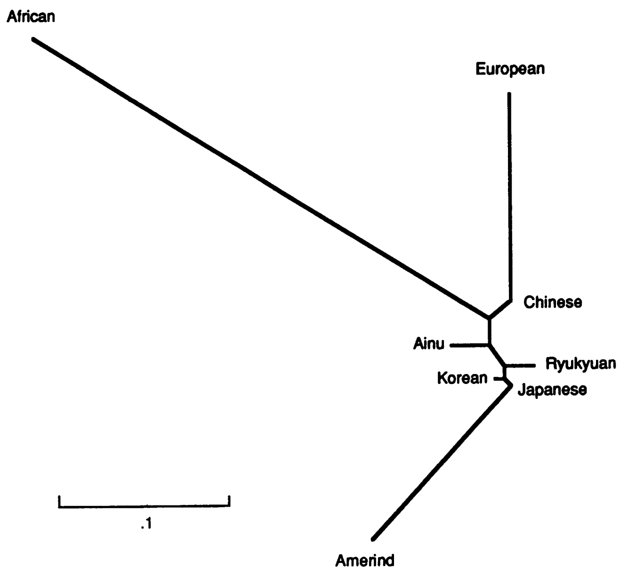

Figure 3 NJ network, showing the relationships of the five East Asian, African, European, and Amerind populations based on d_A (×100) distances.

Conclusion and Perspectives

The nucleotide sequences of mtDNA from 293 East Asians sampled in three Japanese populations (mainland Japanese, Ryukyuans, and Ainu) and two populations outside of Japan (Koreans and Chinese) were analyzed. Through extensive analysis of sequence data, we tried to deduce the genetic background of the three Japanese populations and ask how the present Japanese were formed. On the basis of the sequence data from other East Asian populations outside of Japan, we were able to gain insights into the history of the formation of modern Japanese. Although the mainland Japanese shared some degree of genetic affinity with the Ainu and Ryukyuans, who are considered modern descendants of the aboriginal Jomon people, a great majority of the gene pool in the mainland Japanese was derived from

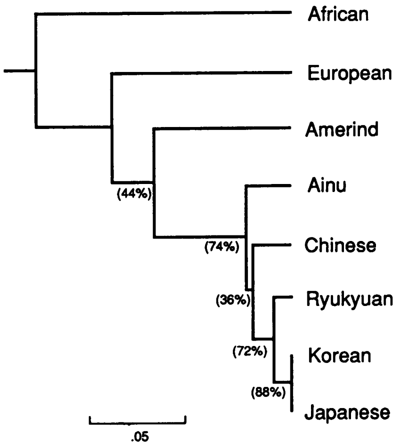

Figure 4 UPGMA phylogenetic tree, showing the relationships of the five East Asian, African, European, and Amerind populations based on d_A (×100) distances. The number in parenthesis for each interior branch is the bootstrap probability (Felsenstein 1985).

the Asian continent through migrations after the Yayoi Age. Thus, the present results lend support to the hybridization theory on the origin of modern Japanese. Furthermore, the Ainu and Ryukyuans, though they shared some genetic affinity with each other, are thought to have existed as different populations when the Yayoi migration began to take place. Phylogenetic analysis of the entire human population revealed a close genetic relationship among East Asian populations. The present study also revealed the unique phylogenetic position of the Ainu, whose genetic origin still remains uncertain. When future studies obtain even more thorough geographic sampling, especially from Southeast Asia and Siberia, we will be able to attain a deeper understanding of the phylogenetic affiliation of the Ainu as modern descendant of the Neolithic Jomon people.

Acknowledgments

We would like to thank Drs. N. Takahata and M. K. Uyenoyama for their comments on this manuscript. This study was supported by grants-in-aid for scientific research on the priority area to S.H. from the Ministry of Education, Science, and Culture, Japan. The sequences reported in this paper have been deposited in the DNA Data Bank of Japan data base (accession nos. D84723–D85015).

References

Anderson SA, Bankier T, Barrell BG, De Bruijn MH, Coulson AR, Sanger F, Schreier PH, et al (1981) Sequence and organization of the human mitochondrial genome. Nature 290: 457–465

Ballinger SW, Schurr TG, Torroni A, Gan Y-Y, Hodge JA, Hassan K, Chen KH, et al (1992) Southeast Asian mitochondrial DNA analysis reveals genetic continuity of ancient Mongoloid migrations. Genetics 130:139–152

Bellwood P, Fox JJ, Tryon D (1995) The Austronesians: historical and comparative perspectives. The Australian National University, Canberra

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution human evolutionary trees with polymorphic microsatellites. Nature 368: 455–457

Brown WM (1980) Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. Proc Natl Acad Sci USA 77:3605–3609

Brown WM, George M Jr, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76: 1967–1971

Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. Nature 325:31–36

Chard C (1974) Northeast Asia in prehistory. University of Wisconsin Press, Madison

Di Rienzo A, Wilson AC (1991) Branching pattern in the evolutionary tree for human mitochondrial DNA. Proc Natl Acad Sci USA 88:1597–1601

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Giles RE, Blanc H, Cann HM, Wallace DC (1980) Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci USA 77:6715–6719

Hagelberg E, Clegg JB (1993) Genetic polymorphisms in prehistoric Pacific islanders determined by analysis of ancient bone DNA. Proc R Soc Lond B 252:163–170

Hammer MF, Horai S (1995) Y chromosomal DNA variation and the peopling of Japan. Am J Hum Genet 56:951–962

Hanihara K (1987) Estimation of the number of early migrants to Japan. J Anthropol Soc Nippon 95:391–403

——— (1991) Dual structure model for the population history of the Japanese. Jpn Rev 2:1–33

——— (1992) Dual structure model for the formation of the Japanese population. In: Hanihara K (ed) Japanese as a member of the Asian and Pacific populations: International Symposium 4. International Research Center for Japanese Studies, Kyoto, pp 245–251

Harihara S, Hirai M, Suutou Y, Shimizu K, Omoto K (1992) Frequency of a 9-bp deletion in the mitochondrial DNA among Asian populations. Hum Biol 66:161–166

Harihara S, Saitou N, Hirai M, Gojobori T, Park KS, Misawa S, Ellepola SB, et al (1988) Mitochondrial DNA polymorphism among five Asian populations. Am J Hum Genet 43: 134–143

Hasebe K (1940) The ancient Japanese. J Anthropol Soc Nippon 55:27–34 (in Japanese)

Hertzberg M, Mickleson KNP, Serjeantson SW, Prior JF, Trent RJ (1989) An Asian-specific 9-bp deletion of mitochondrial DNA is frequently found in Polynesians. Am J Hum Genet 44:504–510

Horai S (1991*a*) A genetic trail of human mitochondrial DNA. In: Mukohata Y (ed) New era of bioenergetics. Academic Press, Tokyo, pp 273–299

——— (1991*b*) Molecular phylogeny and evolution of human mitochondrial DNA. In: Kimura M, Takahata N (eds) New aspects of the genetics in molecular evolution. Japan Scientific Societies Press, Tokyo/Springer, Berlin, pp 135–152

Horai S, Hayasaka K (1990) Intraspecific nucleotide sequence differences in the major noncoding region of human mitochondrial DNA. Am J Hum Genet 46:828–842

Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N (1995) Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proc Natl Acad Sci USA 92:532–536

Horai S, Gojobori T, Matsunaga E (1986) Distinct clustering of mitochondrial DNA types among Japanese, Caucasians, and Negroes. Jpn J Genet 61:271–275

——— (1987) Evolutionary implications of mitochondrial DNA polymorphism in human populations. In: Vogel F, Sperling K (eds) Human genetics: Proceedings of the 7th International Congress. Springer-Verlag, Heidelberg, pp 177–181

Horai S, Kondo R, Murayama K, Hayashi S, Koike H, Nakai N (1991) Phylogenetic affiliation of ancient and contemporary humans inferred from mitochondrial DNA. Philos Trans R Soc Lond [B] 333:409–417

Horai S, Kondo R, N-Hottori Y, Hayashi S, Sonoda S, Tajima K (1993) Peopling of the Americas, founded by four major lineages of mitochondrial DNA. Mol Biol Evol 10:23–47

Horai S, Matsunaga E (1986) Mitochondrial DNA polymorphism in Japanese. II. Analysis with restriction enzymes of four or five base pair recognition. Hum Genet 72:105–117

Hutchison CA III, Newbold JE, Potter SS, Edgell MH (1974) Maternal inheritance of mammalian mitochondrial DNA. Nature 251:536–538

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:112–120

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86: 6196–6200

Kozintsev AG (1993) Ainu origins in the light of modern physical anthropology. Homo 44:105–127

Lum JK, Rickards O, Ching C, Cann RL (1994) Polynesian mitochondrial DNAs reveal three deep maternal lineage clusters. Hum Biol 66:567–590

Mizoguchi Y (1986) Contributions of prehistoric Far East populations to the population of modern Japan: a Q-mode path analysis based on cranial measurements. In: Akazawa T, Aikens CM (eds) Prehistoric hunter gatherers in Japan. University of Tokyo Press, Tokyo, pp 107–136

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York, pp 293–298

——— (1995) The origins of human populations: genetic, linguistic and archeological data. In: Brenner S, Hanihara K (eds) The origin and past of modern humans as viewed from DNA. World Scientific, Singapore, pp 71–91

Nei M, Livshits G (1989) Genetic relationships of Europeans, Asians and Africans and the origin of modern *Homo sapiens*. Hum Hered 39:276–281

Nei M, Miller JC (1990) A simple method for estimating average number of nucleotide substitutions within and between population from restriction data. Genetics 125:873–879

Nei M, Roychoudhury AK (1993) Evolutionary relationships of human populations on a global scale. Mol Biol Evol 10: 927–943

Omoto K (1992) Some aspects of the genetic composition of the Japanese. In: Hanihara K (ed) Japanese as a member of the Asian and Pacific populations: International Symposium 4. International Research Center for Japanese Studies, Kyoto, pp 139–145

——— (1995) Genetic diversity and origins of the "Mongoloids." In: Brenner S, Hanihara K (eds) The origin and past of modern humans as viewed from DNA. World Scientific, Singapore, pp 92–109

Passarino G, Semino O, Modiano G, Santachiara-Benerecetti AS (1993) CoII/tRNA^{Lys} intergenic 9-bp deletion and other mtDNA markers clearly reveal that the Tharus (southern Nepal) have Oriental affinities. Am J Hum Genet 53:609–618

Redd AJ, Takezaki N, Sherry ST, McGarvey ST, Sofro ASM, Stoneking M (1995) Evolutionary history of the COII/tRNA^{Lys} intergenic 9 base pair deletion in human mitochondrial DNAs from the Pacific. Mol Biol Evol 12(4):604–615

Reed TE (1969) Caucasian genes in American Negroes. Science 165:762–768

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi RG, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Saitou N, Nei M (1987) The neighbor-joining method: a new method for constructing phylogenetic trees. Mol Biol Evol 4:406–425

Schurr TG, Ballinger SW, Gan YY, Hodge JA, Merriwether DA, Lawrence DN, Knowler WC, et al (1990) Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. Am J Hum Genet 46:613–623

Shields GF, Hecker K, Voevoda MI, Reed JK (1992) Absence of the Asian-specific region V mitochondrial marker in Native Beringians. Am J Hum Genet 50:758–765

Shields GF, Schmiechen AM, Frazier BL, Redd A, Voevoda MI, Reed JK, Ward RH (1993) mtDNA sequences suggest a recent evolutionary divergence for Beringian and northern North American populations. Am J Hum Genet 53:549–562

Stoneking M, Wilson AC (1989) Mitochondrial DNA. In: Hill A, Serjeantson S (eds) The colonization of the Pacific: a genetic trail. Clarendon Press, Oxford, pp 215–245

Suzuki H (1969) Microevolutionary changes in the Japanese population for the prehistoric age to the present day. J Fac Sci Univ Tokyo Sec V 3:279–308

——— (1981) Racial history of the Japanese. In: Schwidetzkys I (ed) Lieferung Asien I: Japan, Indonesien, Ozeanien. Vol 8 in: Abbie A, Saller K, Schwidetzkys I (eds) Rassengeschichte der Menschhiet. R Oldenbourg, Munich, pp 7–69

Torroni A, Chen Y-S, Semino O, Santachiara-Beneceretti AS, Scott CR, Lott MT, Winter M, et al (1994) mtDNA and Y-chromosome polymorphisms in four Native American populations from southern Mexico. Am J Hum Genet 54:303–318

Torroni A, Schurr TG, Yang C, Szathmary EJE, Williams RC, Schanfield MS, Troup GA, et al (1992) Native American mitochondrial DNA analysis indicates that the Amerind and the Nadene populations were founded by two independent migrations. Genetics 130:153–162

Turner CG (1976) Dental evidence on the origins of the Ainu and Japanese. Science 193:911–913

——— (1990) Major features of sundadonty and sinodonty, including suggestions about east Asian microevolution, population history and late Pleistocene relationships with Australian aboriginals. Am J Phys Anthropol 82:295–317

Vigilant L (1990) Control region sequences from African populations and evolution of human mitochondrial DNA. PhD thesis, University of California, Berkeley

Vigilant L, Pennington R, Harpending H, Kocher TD, Wilson AC (1989) Mitochondrial DNA sequences in single hairs from a southern African population. Proc Natl Acad Sci USA 86 :9350–9354

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC (1991) African populations and the evolution of human mitochondrial DNA. Science 253:1503–1507

Ward RH, Frazier BL, Dew-Jager K, Pääbo S (1991) Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720–8724

Ward RH, Redd A, Valencia D, Frazier B, Pääbo S (1993) Genetic and linguistic differentiation in the Americas. Proc Natl Acad Sci USA 90:10663–10667

Wallace DC (1995) Mitochondrial DNA variation in human evolution, degenerative disease, and aging. Am J Hum Genet 57:201–223

Yamaguchi B (1982) A review of the osteological characteristics of the Jomon population in prehistoric Japan. J Anthropol Soc Nippon 90:77–90